

CLAIMS

Ins. B 3
1. A polynucleotide comprising a transcriptionally-activated Adeno-associated virus (AAV) inverted terminal repeat (ITR), wherein the transcriptionally-activated ITR is less than about 400 bp in length and comprises a heterologous transcriptionally active element, and wherein the transcriptionally-activated ITR exhibits at least about a two-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription.

2. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR is less than about 200 bp.

3. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR exhibits at least about a seven-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription.

4. A polynucleotide according to claim 3 wherein the transcriptionally-activated ITR comprises a transcription initiator sequence and at least one CCAC box.

5. A polynucleotide according to claim 4 wherein the transcription initiator sequence and at least one CCAC box are contained within a polynucleotide segment less than about 90 nt.

6. A polynucleotide according to claim 5 wherein the transcriptionally active element of the transcriptionally-activated ITR has at least about 90% overall identity to SEQ ID NO:17, or the sequence complementary thereto.

7. A polynucleotide according to claim 4 wherein said polynucleotide comprises SEQ ID NO:17.

8. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR exhibits at least about a 10-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription.

5 9. A polynucleotide according to claim 8 wherein the transcriptionally-activated ITR comprises a transcriptionally active element of an amyloid β -protein precursor (APP) promoter and a transcription initiator sequence.

10 10. A polynucleotide according to claim 9 wherein the transcriptionally active element of an amyloid β -protein precursor (APP) promoter and the transcription initiator sequence are contained within a polynucleotide segment less than about 70 nt.

15 11. A polynucleotide according to claim 10 wherein the transcriptionally active element of the transcriptionally-activated ITR has at least about 90% overall sequence identity to SEQ ID NO:7, or the sequence complementary thereto.

20 12. A polynucleotide according to claim 9 wherein said polynucleotide comprises SEQ ID NO:7.

25 13. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR exhibits at least about a 40-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription.

30 14. A polynucleotide according to claim 13 wherein the transcriptionally-activated ITR comprises an ATF-1/CRE site, an Sp1 site and a transcription initiator sequence.

35 15. A polynucleotide according to claim 14 wherein the ATF-1/CRE site, the Sp1 site and the transcription initiator sequence are contained within a polynucleotide segment less than about 85 nt.

16. A polynucleotide according to claim 15 wherein the transcriptionally active element of the transcriptionally-activated ITR has at least about 90% overall sequence identity to SEQ ID NO:11, or the sequence complementary thereto.

5 17. A polynucleotide according to claim 14 wherein said polynucleotide comprises SEQ ID NO:11.

10 18. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR exhibits at least about a 50-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription.

15 19. A polynucleotide according to claim 18 wherein the transcriptionally-activated ITR comprises an ATF-1/CRE site, an Sp1 site, a C box element of the Na,K-ATPase α 1 subunit gene promoter, and a transcription initiator sequence.

20 20. A polynucleotide according to claim 19 wherein the ATF-1/CRE site, the Sp1 site, C box element, and the transcription initiator sequence are contained within a polynucleotide segment less than about 110 nt.

25 21. A polynucleotide according to claim 20 wherein the transcriptionally active element of the transcriptionally-activated ITR has at least about 90% overall sequence identity to SEQ ID NO:13, or the sequence complementary thereto.

25 22. A polynucleotide according to claim 19 wherein said polynucleotide comprises SEQ ID NO:13.

30 23. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR comprises a heterologous transcription initiator sequence.

30 24. A polynucleotide according to claim 1 wherein the transcriptionally-activated ITR comprises a TATA box as a transcription initiator sequence.

25. A polynucleotide comprising, in order:

a first ITR which is a transcriptionally-activated ITR, wherein the transcriptionally-activated ITR is less than about 400 bp in length and comprises a transcriptionally active element, and wherein the transcriptionally-activated ITR exhibits at least a two-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription; and

a second ITR selected from the group consisting of a wild-type ITR, a transcriptionally-activated ITR, a D sequence, a trs, or a portion of a wild-type ITR.

26. A polynucleotide according to claim 25 wherein the transcriptionally-activated ITR is less than about 200 bp.

27. A plasmid comprising a polynucleotide of claim 25, further comprising an element selected from the group consisting of an origin of replication and a reporter gene.

28. A polynucleotide according to any of the previous claims further comprising a gene operably linked to the transcriptionally-activated ITR.

29. A polynucleotide of claim 28, wherein the gene is a CFTR gene.

30. An AAV viral particle comprising a polynucleotide of any of the previous claims.

31. A mammalian cell comprising a polynucleotide according to any of claims 1 to 29, wherein said polynucleotide is stably integrated into a chromosome of said cell.

32. A mammalian cell of claim 31, wherein said cell comprises an AAV *rep* gene and an AAV *cap* gene.

33. A mammalian cell of claim 31, wherein said cell comprises an AAV *rep* gene and an AAV *cap* gene stably integrated into a chromosome of said cell.

34. A method of packaging a recombinant AAV vector, comprising the steps of:

- a) providing a mammalian cell;
- b) introducing a recombinant AAV vector, said vector comprising:

a first ITR which is a transcriptionally-activated ITR, wherein the transcriptionally-activated ITR is less than about 400 bp in length and comprises a transcriptionally active element, and wherein the transcriptionally-activated ITR exhibits at least a two-fold increase in transcriptional activity relative to a wild-type ITR under conditions permissive for transcription;

and a second ITR selected from the group consisting of a wild-type ITR, a transcriptionally-activated ITR, a D sequence, a trs, or a portion of a wild-type ITR;

- c) providing Rep and Cap proteins within the cell;
- d) providing helper virus or helper virus functions; and
- e) incubating the cell under conditions suitable for replication and packaging of the AAV vector.

35. A method according to claim 34, wherein the Rep and Cap proteins are produced from *rep* and *cap* genes integrated into a chromosome of the cell.